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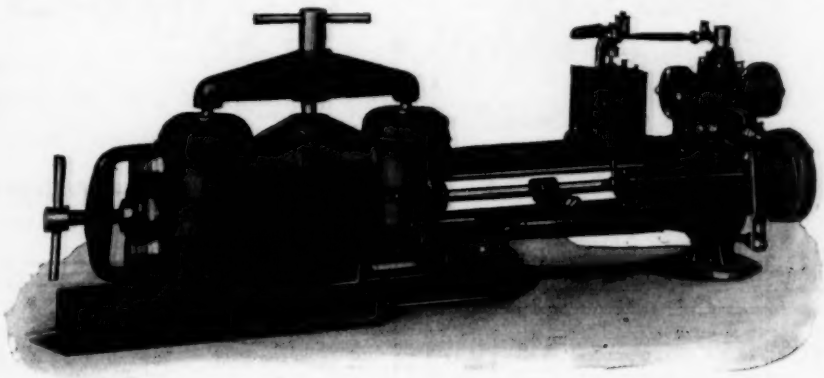

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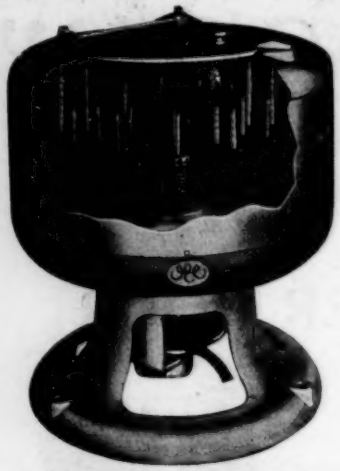

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THE HEAT COAGULATION OF MILK[1]

H. H. SOMMER AND E. B. HART

Departments of Dairy Husbandry and Agricultural Chemistry, University of Wisconsin, Madison, Wisconsin

In the sterilizing process in the manufacture of evaporated milk, it is desirable to produce an incipient coagulation, in the nature of a tender jell or "liver" as it is called by the practical men. Such a product will have a heavy creamy body after the "liver" has been broken up by the shaking process. This is desirable because the product presents a richer appearance to the consumer, and on account of the greater viscosity, there is little danger of fat separation from such a product.

However, evaporated milk differs widely in the readiness with which it coagulates. At times the milk coagulates so readily that it would be unmarketable, on account of its curdy appearance, if it were heated long enough to preserve it. This constitutes the problem with which we are concerned in this paper.

There is probably no condensery that is entirely free from this trouble throughout the year. It is undoubtedly one of the biggest problems that confronts the industry.

SUMMARY OF PREVIOUS ARTICLE

In a previous article (1) this problem was investigated indirectly in a study of the factors that influence the heat coagulation of fresh milk at 136°C. The following conclusions were based on this study:

1. In fresh milk there is no relation between the titratable acidity and the heat coagulation.

2. The hydrogen ion concentration of fresh milk is not the determining factor in the heat coagulation.

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

3. The concentration of the milk influences the heat coagulation, but it accounts only partly for the wide differences in the heat coagulation of fresh milk samples.

4. The main factor in the heat coagulation of fresh milk is the salt composition of the milk, especially the calcium, magnesium, citrate and phosphate content. The effect of the calcium and magnesium salts is opposed to the effect of the citrates and phosphates and vice versa. An excess of either of these two classes of salts causes the milk to coagulate more readily; the proper balance produces the most stable condition.

Since the publication of the previous paper the above conclusion on the importance of the milk salts has been substantiated by a study of a large number of samples of fresh milk from a number of different cows. Whenever a sample was found that coagulated at 137 to 138°C.[2] the effect of additions of sodium citrate, di-potassium phosphate, calcium acetate, and sodium bicarbonate was observed. Sodium bicarbonate was included because it is so commonly used in evaporated milk.

In all cases it was possible to prevent the coagulation by a small addition of salts normally present in milk. Table 1 gives a few typical results selected from a large number.

Table 1 gives the coagulation of the milk samples under the addition of the solutions of salts in amounts ranging from 0.0 to 0.4 cc. per 25 cc. of milk. In all cases the dilution was equalized by the addition of the proper amount of distilled water.

It will be noted from a study of table 1 that in sample 1 the addition of sodium citrate, sodium phosphate, and sodium bicarbonate prevented the coagulation. The addition of a soluble calcium salt hastened it. In the other four samples the opposite is true; the calcium acetate prevented the coagulation and sodium citrate, sodium phosphate and sodium bicarbonate either did not improve it or actually hastened it.

The action of the sodium bicarbonate is similar to that of the citrates and phosphates; it differs from them in that larger addi-

[2] The xylol used in the xylol vapor bath in the previous paper boiled at 136°C. In this paper the xylol used boiled at 137 to 138°C. This difference is due to differences in purity.

TABLE 1

The influence of salts on the heat coagulation of fresh milk

SAMPLE	25 CC. OF MILK PLUS:	cc. of water	0.0	0.4	0.3	0.2	0.1	0.0
		cc. of solution	0.0	0.0	0.1	0.2	0.3	0.4
			Coagulation at 137°C. in minutes					
1. Clothilde, 11/20/20	Sodium citrate M/4		2:45	3:15	3:30	20:00–	20:00–	6:00
	Sodium phosphate M/4		2:45	3:15	20:00–	20:00–	20:00–	20:00–
	Calcium acetate M/4		3:00	3:15	3:00	1:00	0:30	0:15
	Sodium bicarbonate M/2		3:00	3:15	4:00	20:00–	20:00–	20:00–
2. Wingra, 11/23/20	Sodium citrate M/4		2:00	2:00	2:00	1:45	1:45	1:45
	Sodium phosphate M/4		2:00	2:00	2:00	1:55	1:45	1:45
	Calcium acetate M/4		2:00	2:00	2:30	3:00	20:00–	20:00–
	Sodium bicarbonate M/2		2:00	2:00	2:00	2:00	2:00	2:00
3. Lilac, 12/3/20	Sodium citrate M/4		3:30	3:30	2:00	2:00	1:45	1:45
	Sodium phosphate M/4		3:30	3:30	2:00	2:00	1:45	1:45
	Calcium acetate M/4		3:30	4:00	4:00	20:00–	20:00–	20:00–
	Sodium bicarbonate M/2		3:30	4:00	1:15	1:30	1:30	10:00
4. Lady, 12/2/20	Sodium citrate M/4		2:45	2:45	2:45	2:45	2:30	2:30
	Sodium phosphate M/4		2:45	2:45	2:45	2:30	2:00	2:00
	Calcium acetate M/4		3:00	3:00	4:00	20:00–	20:00–	20:00–
	Sodium bicarbonate M/2		3:00	3:00	2:00	2:00	2:00	20:00–
5. Lily, 11/24/20	Sodium citrate M/4		3:15	3:15	2:45	2:15	2:00	2:00
	Sodium phosphate M/4		3:15	3:15	2:30	2:30	2:30	2:30
	Calcium acetate M/4		3:15	3:15	20:00–	20:00–	20:00–	4:00
	Sodium bicarbonate M/2		3:15	3:15	2:15	2:30	20:00–	20:00–

tions prevented the coagulation even where smaller additions hastened it. This indicates that the influence of the sodium bicarbonate is twofold—it has a balancing effect on the calcium, and it changes the reaction. On this basis the results obtained with samples 2, 3, 4 and 5 can be explained as follows:

These samples coagulated because of a low content of calcium. The sodium bicarbonate tends to increase this deficiency by counteracting the effect of the calcium present; as a result the coagulation is hastened. However, with the larger additions of the sodium bicarbonate the reaction of the milk is changed to such an extent that the casein is held in solution, the sodium probably replacing part of the calcium in the casein.

EVAPORATED MILK

The underlying idea in the work on the heat coagulation of fresh milk was that the factors that influence the coagulation of fresh milk would also be applicable to evaporated milk. The demonstration that the salts which are normally present in milk have a very decided effect on the stability of the casein in fresh milk is quite conclusive. This logically leads to the conclusion that they must have a similar effect on the evaporated milk, for we are here dealing with an entirely similar system, modified only to the extent to which it has been concentrated and the heat treatment and other manipulations it has undergone. The experiments carried on with evaporated milk and the observations made under commercial conditions verify this conclusion.

On account of the small amounts of the normal milk salts that affect the heat coagulation, their effect on evaporated milk was studied by adding them to the milk, using small amounts well within the limits of normal variations in milk. A number of such experiments were made on evaporated milk produced under commerical conditions. In most cases the salt solutions were added to the milk in the can, the can sealed, shaken and immediately sterilized. In several cases their effect was studied when added before the milk was concentrated. The results are given in tables 2, 3, 4 and 5.

TABLE 2

Evaporated milk from condensery A, December 29, 1920

16 OUNCES OF EVAPORATED MILK PLUS:	cc. of water	0.0	12.0	9.0	6.0	3.0	0.0
	cc. of solution	0.0	0.0	3.0	6.0	9.0	12.0
Calcium acetate M/4		3*	3	3+	4	5	6
Sodium phosphate M/4		3	3	2	2−	1	1
Sodium bicarbonate M/2		3	3	3−	2+	2	1

* Indicates the relative firmness of the coagulum.

TABLE 3

Evaporated milk from condensery B, December 29, 1920

6 OUNCES OF EVAPORATED MILK PLUS:	cc. of water	0.0	4.0	2.0	0.0
	cc. of solution	0.0	0.0	0.2	0.4
Sodium citrate M/4		1*	1	1	2
Calcium acetate M/4		1	1	4	5
Sodium phosphate M/4		1	1	2	3
Sodium bicarbonate M/2		1	1	3	4

* The figures indicate the relative firmness of the coagulum.

TABLE 4

Evaporated milk from condensery C, March 5, 1921

6 OUNCES OF EVAPORATED MILK PLUS:	cc. of water	0.0	3.0	2.5	2.0	1.5	1.0	0.5	0.0
	cc. of solution	0.0	0.0	0.5	1.0	1.5	2.0	2.5	3.0
Sodium phosphate M/4		2S*	1S	0+S	0+R	0+R	0+R	0+R	0+R
Sodium citrate M/4		2S	1S	1S	0+S		0+S	0S	0S
Calcium acetate M/4		2S	1S	2S	3S	3+S	4R	4+R	5R
Sodium bicarbonate M/2		2S	1S	0S	0+S	0+S	1+S	1+S	2S

* The numerals indicate the relative firmness of the coagulum. S indicates that the body of the milk was smooth on shaking. R indicates that the body of the milk was rough on shaking.

TABLE 5

Evaporated milk from condensery D, March 13, 1921

16 OUNCES OF EVAPORATED MILK PLUS	cc. of water	0.0	6.0	4.5	3.0	1.5	0.0
	cc. of solution	0.0	0.0	1.5	3.0	4.5	6.0
Sodium citrate M/4		2S*	2−S	1+S	1S	1−S	1−S
Potassium phosphate M/4		2S	2−S	2−R	1+R	1R+	1−R++
Calcium acetate M/4		2S	2−S	3R	4R	5R+	6 (whey)
Sodium bicarbonate M/2		2S	2−S	1+R	1+S	1−S	1S

* Same as in table 4.

The results in tables 2, 3, 4 and 5 show that the addition of sodium citrate, sodium phosphate, calcium acetate and sodium bicarbonate has the same effect on the evaporated milk as on the fresh milk and casein solutions. The observations in table 2 were made in an attempt to increase the process at a condensery that had considerable trouble with the coagulation. The results showed that both sodium phosphate and bicarbonate improved the milk. Sodium bicarbonate added to the milk greatly increases the caramelization and impairs the flavor, as a result its further use was not advisable. Sodium citrate had not been tried in this case but its effect always is similar to that of the phosphate, and since we had only sodium citrate on hand in sufficient amounts, its use was recommended in this case. Two ounces of sodium citrate were added per 1000 pounds of raw milk, and this increased the sterilizing process four minutes at 240°F. This addition which amounted to an increase of 0.0067 per cent in the citric acid content of the milk changed the sterilizing process from an unsafe to a satisfactory process.

The results in tables 3, 4 and 5 were gathered merely as a demonstration; they had no immediate practical application since the evaporated milk in all three cases was entirely satisfactory without any addition. The fact that sodium bicarbonate, which is so commonly used, can have an injurious effect on the process is shown in tables 3 and 4.

The effect of calcium chloride on the coagulation of the evaporated milk was accidently demonstrated at condensery D when a small amount of calcium chloride brine leaked into the milk at the aerater. This small amount of calcium chloride, not enough so that the analysis of the milk showed an abnormal content of calcium, caused the milk to coagulate so severely even at the minimum sterilizing process that it would not flow out of the open can when it was inverted. By adding the proper amount of sodium citrate, within the limits of normal variation of this constituent in milk, and heating up the milk by drawing it through the vacuum pan, the product finally processed at 244°F. for sixteen minutes. It was entirely normal in appearance, taste and analyses. This was a striking illustration on a large

scale (50,000 pounds of milk) that the salts have a very marked effect on the coagulation of the evaporated milk.

The beneficial effect of sodium citrate and di-sodium phosphate has been demonstrated in a number of other plants, according to reports from condenseries that had trouble with low sterilizing processes, and where the use of these salts had been recommended. One company operating several condenseries writes,

> We have been trying out the di-sodium phosphate with marked success. In some instances it has enabled us to extend our (sterilizing) process four or five minutes and it has consistently given us two or three minutes of extra time. . . . . We have saved ourselves a great deal of trouble by using this method.

Thus it is demonstrated that the salt balance is of importance in the manufacture of evaporated milk. The process of adjusting the salt balance in order to avoid low sterilizing processes is already an accepted commercial practice in a number of plants as a result of this study.

The conclusion that the milk salts have a decided effect on the heat coagulation of evaporated milk is contrary to the conclusion of Rogers, Deysher and Evans (2). In their study of the factors involved in the coagulation of evaporated milk they concluded that there is no definite relation between the "acid-base ratio" and the coagulation and that the composition of the milk salts is "only a minor factor in determining the coagulating temperature of evaporated milk." As their criterion in arriving at this conclusion they applied the analytical method used by us in our previous paper. The milk samples were analyzed for calcium, magnesium, citric acid and phosphoric acid, and the excess of calcium and magnesium in gram equivalents over the citric acid and phosphoric acid was calculated. Using this method as a criterion the samples having the largest excess of calcium and magnesium should coagulate most readily. Rogers, Deysher and Evans failed to find this relation and based their conclusion entirely on this fact.

Their conclusion undoubtedly is erroneous because of the inadequacy of the analytical method which they used as their criterion. In our previous paper this method was applied to

fresh milk not as the sole test but simply as an interesting analytical verification of a fact which had already been amply demonstrated synthetically. We found only a general agreement between the excess of calcium and magnesium and the coagulability of fresh milk. There are several considerations which can readily account for any irregularities:

1. The analyses deal with the total content of the salts while only that portion of the salt remaining in solution as the temperature is raised affects the coagulation.

2. The amounts of the salts necessary to affect the coagulation are very small, within or approaching the limits of experimental error of the analytical methods.

3. The calculations are based only on approximate results as to the equivalency of the salts.

4. Other factors such as concentration and reaction are not taken into consideration.

These considerations which cause only a general instead of a detailed agreement between the calculated excess of calcium and magnesium and the coagulation of fresh milk, become of even greater moment in attempting to apply this calculation to evaporated milk. The extent to which the salts have been changed to an insoluble form is greater in evaporated milk, and the reaction of evaporated milk is more acid so that the equivalency of the salts may be different. As a result it is not surprising to find that this method used as the sole criterion leads to erroneous conclusions.

THE ACTION OF THE MILK SALTS IN THE COAGULATION

It has been suggested that the addition of the salts changes the reaction of the milk and that the action of the salts in preventing the coagulation is to be attributed to this rather than to a direct influence of the salts on the stability of the casein.

Zoller (3) in working with solutions of milk salts found that the solutions were more acid in reaction after heating and that the change in reaction was proportional to the amount of calcium precipitated. He found further that the amount of calcium

precipitated and the change in acidity was greatly reduced by doubling the citrate content of the salt solutions. The conclusion from this work appears to be that the beneficial effect of sodium citrate in preventing the heat coagulation of milk is to be attributed to its indirect effect on the reaction. That this explanation is inadequate is demonstrated by the following experiments.

The change in reaction caused by the addition of a salt to the milk may be considered in three different ways:

1. The salt added to the milk at room temperature may change the reaction due to the akalinity or acidity of the salt or to its buffer effect.

2. If the milk is heated after the salt has been added there may be a change in reaction attributable to the salt, due to interaction of the milk salts and precipitation of calcium phosphate.

3. The reaction may be considered in a dynamic sense. It is a well-known fact that the dissociation constant of water varies very decidedly with the temperature. In a similar manner the dissociation constants of the various milk salts vary with the temperature. The final reaction that results in the milk at any given temperature is determined by the equilibrium which obtains between all the constituents, each with its characteristic dissociation constant. As a result there is the possibility that two samples of milk of the same reaction at room temperature, but of a different composition, may have reactions differing from each other at higher temperatures. In a complex substance such as milk it is impossible to calculate what this change in reaction with a given change in temperature will be. However, it is probable that there may be an appreciable difference in the reaction of two milk samples having the same reaction at room temperature.

That the beneficial effect of the addition of a salt to the milk is not to be attributed to a change in reaction in the first and second sense as outlined above is demonstrated by the following experiments (tables 6 and 7).

A sample of heat coagulable milk from an individual cow of the University herd was selected. The reaction of this milk was

studied both before and after the addition of calcium acetate in sufficient amounts to prevent the coagulation. The results are given in table 6.

The coagulation of both samples in table 6 was prevented by the addition of calcium acetate. The results show that there was little or no change in the reaction accompanying the addition of the salt to the milk at room temperature, so that the beneficial effect cannot be attributed to a change in reaction in this sense. After the samples had been heated at 100°C. for twenty minutes the samples that had the calcium acetate added were slightly more acid than those that were heated similarly without the calcium acetate present. However, the difference was so slight

TABLE 6

The change in reaction accompanying the addition of calcium acetate to the milk

	625 cc. of milk plus:			
	Sample 1, February 15, 1921		Sample 2, March 1, 1921	
	cc. of distilled water	cc. of calcium acetate	cc. of distilled water	cc. of calcium acetate
Heat coagulation at 137°C	2:30	20:00–	2:30	20:00–
Reaction at room temperature, pH	6.59	6.59	6.60	6.58
Reaction at room temperature after heating at 100°C. for twenty minutes, pH	6.53	6.48	6.51	6.48
Change in reaction due to heating pH	0.06	0.11	0.09	0.10

that very little importance can be attached to it. As will be shown further in table 7 the beneficial effect of the calcium acetate cannot be attributed to this slight increase in acidity.

Sample 2 was one that was actually benefited by an increase in acidity but the increase necessary was much greater than that obtained in the case of adding calcium acetate. This is shown by the following experiment: The sample of milk was divided into two portions, one was kept at a temperature near the freezing point, and the other portion was placed in the incubator and the acidity allowed to develop to 0.65 per cent. A series of samples of varying acidity were prepared by mixing the two portions in varying proportions. The coagulation of the samples was studied

and the reaction of two samples determined. The results are given in table 7.

The results given in table 7 show that in this sample an increase of acidity prevented the coagulation, but the reaction at which this prevented the coagulation was much more acid than the reaction obtained under the optimum addition of calcium acetate as shown in table 6. This indicates that the calcium acetate (see table 6) had an effect as such and not by virtue of changing the reaction.

TABLE 7

Heat coagulation prevented by the increase in acidity

TUBE NUMBER	9 CC. OF MILK* (ACIDITY 0.15 PER CENT) PLUS SOUR MILK (ACIDITY 0.65 PER CENT)	ACIDITY OF MIXTURE LACTIC ACID	COAGULATION AT 137°C.	pH
	cc. of sour milk	*per cent*		
1	0.0	0.150	2:30	6.60
2	0.1	0.155	5:00	
3	0.2	0.160	6:00	
4	0.3	0.166	20:00—	
5	0.4	0.171	20:00—	6.23
6	0.5	0.176	20:00—	
7	0.6	0.182	20:00—	
8	0.7	0.187	0:45	
9	0.8	0.193	0:30	
10	0.9	0.198	0:15	

* This is the same sample of milk as sample 2 in table 6.

The question of whether or not the action of the salts in preventing the heat coagulation is to be explained by their influence on the reaction in the dynamic sense cannot be answered directly, for it is impossible to measure the reaction at the high temperature at which the heat coagulation takes place. However, the question can be answered indirectly by demonstrating that the milk salts have a very decided effect on the stability of the casein under somewhat different conditions.

It has been shown by Van Slyke and Hart (4) and by Van Slyke and Bosworth (5) that calcium chloride has a marked effect on the solubility of casein in lime water. Their work showed

that in neutralizing a lime water solution of casein with hydrochloric acid the neutralization could be carried much farther without the precipitation of the casein if the calcium chloride formed was removed by dialysis. The calcium chloride has the effect of precipitating the casein.

If the citrates and phosphates counteract the effect of the calcium in the heat coagulation, then they should show a similar action in preventing the precipitation of the casein at room temperature. As a preliminary experiment to demonstrate this, 4 grams of purified casein were dissolved in 100 cc. of 0.9737 N/20 calcium hydroxide solution. To portions of this solution hydrochloric acid, sodium phosphate, and sodium citrate were added at room temperature. The results are given in table 8.

From a study of table 8 it is seen that the citrates and phosphates counteract the effect of the soluble calcium salts on the precipitation of the casein. If the soluble calcium salts are properly balanced with the citrates or phosphates then the casein is held in solution even in an acid reaction (trials 6, 9, 12, 13 and 14). In the absence of citrates and phosphates the casein is precipitated even in alkaline solutions (trials 3 and 4). In trial 3 the addition of calcium acetate to the solution of casein in limewater causes the partial precipitation of the casein. In trial 4 the small amount of calcium chloride formed early in the neutralization with hydrochloric acid causes the partial precipitation of the casein.

If now we neutralize the excess of lime water of the casein solution by means of an N/10 hydrochloric acid solution that contains dissolved in it enough citrate to make it also a M/20 sodium citrate solution, we then shall always have a molecule of sodium citrate present for every molecule of calcium chloride that is formed in the neutralization.

To demonstrate that the sodium citrate does not keep the casein in solution by affecting the reaction, a solution of casein in limewater was neutralized by means of the hydrochloric acid-sodium citrate solution with vigorous stirring until the casein just started to precipitate out. The reaction of the solution was found to be pH 4.81. This demonstrates conclusively that the

TABLE 8

The effect of milk salts on the precipitation of casein from solution

TRIAL NUMBER	10 CC. OF CASEIN SOLUTION PLUS:	RESULT	REACTION TO LITMUS
1	1 cc. sodium phosphate M/4	Solution remains clear	Alkaline
2	1 cc. sodium citrate M/4	Solution remains clear	Alkaline
3	1 cc. calcium acetate M/4	Cloudy—casein precipitates	Alkaline
4	2 cc. HCl N/10	Cloudy—casein precipitates	Alkaline
5	No. 4 plus 1 cc. Na Citrate M/4	Solution clears up	Alkaline
6	No. 5 plus 2 cc. HCl N/10	Solution remains clear	Acid
7	1 cc. Na citrate M/4, 1 cc. Ca acetate M/4 plus 2 cc. HCl N/10	Solution very milky	Alkaline
8	No. 7 plus 1 cc. Na Citrate M/4	Solution clears up	Alkaline
9	No. 8 plus 2 cc. HCl N/10	Solution remains clear	Acid
10	No. 3 plus 1 cc. Na citrate M/4	Casein redissolves	Alkaline
11	No. 4 plus 1 cc. Na_2HPO_4 M/4	Casein redissolves	Alkaline
12	1 cc. Na_2HPO_4 M/4 and 4 cc. HCl N/10	Solution remains clear	Acid
13	No. 12 plus 1 cc. Ca acetate M/4	Casein precipitates	Acid
14	No. 13 plus 1 cc. Na_2HPO_4 M/4	Solution clears up	Acid

sodium citrate has an effect as such and does not keep the casein in solution by its effect on the reaction. The reaction was actually changed to pH 4.81, very close to the isoelectric point for casein before any precipitation took place.

The above experiments with casein solutions dealt entirely with their stability at room temperature. To demonstrate the effect of the sodium citrate on the stability of the casein in the heat coagulation, two series of casein lime water solutions were prepared. One series was neutralized to various stages with N/10 hydrochloric acid, the other series with one N/10 hydrchloric acid—M/20 sodium citrate solution. The dilution in all the samples in the two series was equalized by the addition of distilled water. The samples were subjected to the heat test, and from

TABLE 9

The effect of sodium citrate on the heat coagulation of a casein-lime water solution

25 CC. OF CASEIN SOLUTION PLUS:			HEAT COAGULATION	REACTION pH
Acid solution		cc. of water		
Kind	Amount			
	cc.			
N/10 HCl	1.0	7.0	0:10*	7.64
N/10 HCl, M/20 sodium citrate	8.0	0.0	20:00–	5.48

* Instantly.

each series the proper sample selected for the determination of the hydrogen-ion concentration. The results are given in table 9.

The casein solution in table 9 did not have the sodium citrate present coagulated instantly although its reaction was pH 7.64, while the solution that had the sodium citrate present did not coagulate at 137°C. in twenty minutes at a reaction of pH 5.48. With such a wide difference in the reaction and the behavior of the samples toward heat, we must attribute a direct effect to the sodium citrate that accomplished this great difference.

The effect of the normal milk salts on the coagulation is further emphasized by a study of the effect of sodium citrate and calcium chloride on the coagulation of the casein dissolved in sodium hydroxide (table 10), the effect of sodium phosphate and calcium

TABLE 10

The effect of sodium citrate and calcium chloride on the coagulation of casein solutions

cc. N/4 sodium citrate	5 cc. of casein solution plus: M/4 calcium chloride										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.0	--	x	1:00	0:40 xx	0:30 xx	0:20 xx	xxx	xxx	xxx	xxx	xxx
0.1	--	--	x	1:00	0:50 xx	0:30 xx	0:20 xx	xxx	xxx	xxx	xxx
0.2	--	--	--	x	0:30	0:30 xx	0:20 xx	xxx	xxx	xxx	xxx
0.3	--	--	--	--	x	0:40	0:30 xx	0:25 xx	xxx	xxx	xxx
0.4	--	--	--	--	--	5:00	0:25	0:20 xx	xxx	xxx	xxx
0.5	--	--	--	--	--	3:30	3:00	0:25	0:15 xx	xxx	xxx
0.6	--	--	--	--	--	3:30	3:00	1:00	0:25	0:15 xx	xxx
0.7	--	--	--	--	--	3:30	1:30	1:20	0:50	0:30	0:15 xx
0.8	--	--	--	--	--	3:30	1:30	1:20	1:00	0:50	0:40
0.9	--	--	--	--	--	4:00	1:30	1:20	1:00	0:50	0:40
1.0	--	--	--	--	--	20:00	2:00	1:00	0:50	0:40	0:30

* The casein solution consists of 40 grams of purified casein dissolved in 1 liter of N/10 NaOH, and then neutralized with 700 cc. of 1.042 N/10 NCl, to a reaction of pH 6.60. (The dilution was equalized in all cases with water.)

--, Clear after heating at 137°C. for twenty minutes.

x, Cloudy after heating at 137°C. for twenty minutes.

xx, Cloudy at room temperature before heating.

xxx, Coagulated at room temperature before heating.

chloride on the coagulation of the sodium hydroxide-casein solution (table 11), and the effect of sodium citrate, sodium phosphate and calcium acetate on the coagulation of the casein-lime-water solution (table 12). The results obtained with these solutions where the conditions are less complex than in the milk, are entirely in accord with those obtained with the milk itself.

TABLE 11

The effect of sodium phosphate and calcium chloride on the coagulation of casein solution

5 cc. of casein solution* plus:											
cc. M/4 POTASSIUM PHOSPHATE	cc. M/4 CALCIUM CHLORIDE										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.0	—	—	xx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.1	—	—	—	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.2	—	—	—	—	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.3	—	—	—	—	0:10	xxx	xxx	xxx	xxx	xxx	xxx
0.4	—	—	—	—	—	0:10	xxx	xxx	xxx	xxx	xxx
0.5	—	—	—	—	—	—	xxx	xxx	xxx	xxx	xxx
0.6	—	—	—	—	—	—	xxx	xxx	xxx	xxx	xxx
0.7	—	—	—	—	—	—	xxx	xxx	xxx	xxx	xxx
0.8	—	—	—	—	—	—	0:10	xxx	xxx	xxx	xxx
0.9	—	—	—	—	—	—	—	0:10	xxx	xxx	xxx
1.0	—	—	—	—	—	—	—	—	0:10	xxx	xxx

* The casein solution consists of 40 grams of purified casein dissolved in 1 liter of N/10 NaOH, and then neutralized with 700 cc. of 1.042 N/10 NCl, to a reaction of pH 6.60. (The dilution in each case is equalized by the addition of water.)

xxx, See table 10.

xx, See table 10.

—, See table 10.

If in tables 10 and 11 we to draw a diagonal from the upper left to the lower right hand corner and divide the table each into two fields, we find that in the upper where the calcium chloride is in excess the coagulation takes place most readily, and in the lower field where the sodium citrate (table 10) and the Phosphate (table 11) are in excess the casein solutions do not coagulate at all. The diagonal should be the boundary between these two extremes. With the larger additions of sodium citrate and cal-

cium chloride in table 10 the experimental results deviate from this expected result. The explanation probably is that the higher concentration itself has an effect on the coagulation. In

TABLE 12

The effect of sodium citrate, potassium phosphate, and calcium acetate on the coagulation of casein-lime-water solution

	20 cc. of casein solution* plus:			Heat coagulation at 137°C.
	cc. of 5 per cent Na citrate	cc. of 5 per cent Ca acetate	cc. of water	
Sodium citrate calcium acetate balance	0.00	0.00	0.00	20:00–
	0.75	0.00	0.50	20:00–†
	0.75	0.10	0.40	20:00–†
	0.75	0.20	0.30	20:00–
	0.75	0.30	0.20	20:00–
	0.75	0.40	0.10	2:00
	0.75	0.50	0.00	0.15
	cc. of 5 per cent K_2HPO_4	Ca acetate cc. 5 per cent	cc. of water	Heat coagulation at 137°C.
Di-potassium phosphate calcium acetate balance	0.00	0.00	0.00	20:00–
	1.25	0.00	3.75	18:00‡
	1.25	0.10	3.65	19:00‡
	1.25	0.20	3.55	20:00‡
	1.25	0.30	3.45	20:00‡
	1.25	0.40	3.35	20:00‡
	1.25	0.50	3.25	20:00‡
	1.25	0.60	3.15	0.30
	1.25	0.70	3.05	0.15
	1.25	0.80	2.95	0.15

* The casein solution consisted of 20 grams of purified casein dissolved in 500 cc. of 0.98738 N/20 lime water, neutralized to a reaction of pH 6.68 with 80 cc. of N/10-HCl-M/20 Na citrate and diluted to 800 cc.

† Granular precipitate.

‡ These samples did not show a pronounced precipitate until they were cooled; the precipitate seemed to form immediately then. Similar results were obtained when this experiment was repeated.

table 11 the results are quite sharp, either a sample did not coagulate at all or else it coagulated at room temperature. In tables 10 and 11 where we are dealing with casein-sodium hydroxide solutions, the excess of citrate or phosphate did not cause a

coagulation as they do in milk, but in table 12 where we are dealing with casein-lime water solutions the citrate and phosphate in excess caused a coagulation similar to that caused in milk by their excess.

Although these salts have a very decided effect on the coagulation of the evaporated milk when they are added to the milk after it has been concentrated, the result obtained in that manner are probably not fully indicative of what their effect would have been if they had been normally present in the milk or added before it was concentrated. A more thorough distribution is insured and the interaction of the salts is favored if they are present during the entire process of manufacture. It is common knowledge in the industry that forewarming to a high temperature or holding the milk longer at the high temperature has a beneficial effect in preventing the coagulation. This is undoubtedly due to the interaction of the salts, especially the precipitation of calcium, which is favored by this treatment. If the addition of a salt is to have its fullest effect it must be added to the milk before it is concentrated.

The results obtained under commercial conditions at condensery C will illustrate this, although the observations could not be made under as carefully controlled conditions as would be desirable. For a period of over a month starting early in June, the milk at this condensery coagulated very readily. Careful grading of the milk and rejecting the poor milk, did not remedy the trouble. Sodium bicarbonate only improved the milk slightly. The trials with the additions of sodium citrate and the sodium phosphate to the evaporated milk immediately before it was sterilized showed little improvement. However, the milk already had 3 ounces of sodium bicarbonate added per 1000 pounds of raw milk. The sterilizing process was twelve minutes at 240°F. In order to give the addition of sodium phosphate a fair trial, the use of the soda was discontinued and instead 1 ounce of sodium phosphate added per 1000 pounds of raw milk. This increased the sterilizing process from twelve minutes as it had been the previous day to sixteen minutes at 240°F. There had been no change in the weather conditions and the process had been around

twelve minutes at 240°F. consistently for a number of days, so that it is quite safe to assume that the improvement was due to the difference of adding 1 ounce of sodium phosphate instead of 3 ounces of soda. There is the possibility that the mere elimination of the soda may have been beneficial, but repeated tests had shown that more soda could have been added without injurious effect. While several factors might have contributed to this change the indication is that the sodium phosphate when added to the milk at the forwarmers showed an appreciable improvement, where it had shown little improvement when added to the milk after it had been concentrated.

SUMMARY AND CONCLUSIONS

1. The main factor in the heat coagulation of fresh milk is the salt composition of the milk.

2. The salt composition of the milk is an important factor in the heat coagulation of evaporated milk.

3. In a number of cases the troublesome coagulation of evaporated milk has been remedied on a commercial scale by the addition of the proper amount of sodium citrate or di-sodium phosphate.

4. The milk salts affect the coagulation of milk, both fresh and evaporated, directly. Their effect is not indirect through a change in reaction.

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THE SEASONAL VARIATIONS OF THE PER CENT OF FAT IN COW'S MILK

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The relation between the season of the year and the percentage of fat in milk was shown by Eckles (1) from a study of 240 lactation periods of cows in the Missouri and Iowa Experiment Station herds. He found that regardless of when the lactation began, the per cent of fat when plotted followed a general curve for the year, being lowest during June and July and gradually rising to the highest point in December and January and then again declining until mid-summer. The curves were found to be modified by the evident tendency for the percentage of fat to rise materially during the last two or three months of the milking period. White (2) found that the same relation exists between the solids-not-fat. From a study of the records of the Pacific Creamery and of cows in thirty herds in cow testing associations in the Salt River Valley, Arizona, Clothier (3) concluded that the most probable cause of the difference in fat content of milk between the winter and summer months was the difference in the nature of the feed.

The object of this paper is to present additional data showing the seasonal variations of the per cent of fat in cow's milk.

The data presented in the tables and charts were derived from a study of 3763 Guernsey, 299 Jersey and 95 Holstein-Friesian records.

The Guernsey records were secured from the Advanced Register of Guernsey Cattle. The animals used were representative with respect to their ability as producers except as the minimum requirement of the Advanced Register excludes low producers. The animals were scattered over the United States to some extent but a majority of the records were made in the east and

central western states. These records, therefore, were made under varying conditions from the standpoint of climate, feed, and management.

The Jersey records consist of Register of Merit tests of Jersey cattle made in Missouri during the past few years. Animals of varying producing ability (except those unable to meet the minimum requirements of the Register of Merit) are represented. In this case climatic conditions were similar, being restricted to one state.

The Holstein-Friesian records were secured from the Advanced Register and private records of the Holstein-Friesian herd owned by the University of Missouri. Animals of widely varying productive ability are represented since all cows completing yearly records were included. All cows were under similar herd conditions.

It should be noted that, although varying from year to year, these data include separate records of three breeds made under varying climatic conditions. The Guernsey data is of a national character; the Jersey data is confined to a single state (Missouri); while the Holstein-Friesian data is confined to a single herd.

The records of each breed were divided into groups according to the month during which the lactation began, i.e., all cows calving in January were placed together, those in February together, etc. Records made during parts of two lactation periods were eliminated. The average per cent of fat for each month was obtained by totalling the percentages of fat and dividing this total by the number of cows in the group. This method was used rather than the so-called "true average" per cent obtained by dividing the total of the fat by the total of the milk due to the fact that the first method is used by the various breed associations in obtaining the average per cent of fat when several separate tests are made on a cow during a single month. To be consistent the same method was used in our work. A comparison was made of the two methods, however, and quite similar results were obtained.

By grouping the records according to the month during which the lactation began the influence of the stage of lactation as a

factor in the variation of the per cent of fat in the milk can be shown in conjunction with seasonal variations. In a previous paper (4) we presented values derived from the same data showing the influence of the advance of lactation on the per cent of fat. Theoretically, if there were no other factors affecting the per cent of fat in cow's milk during a lactation period, the values obtained for the average per cent of fat by each group beginning the lactation period during the various months would follow the same general curve as the values derived without consideration of the month when the lactation began. A significant difference was found when these two values were plotted together.

The data for the Guernsey breed is presented in table 1 and chart 1. The continuous lines in the charts are the plotted values showing the influence of the stage of lactation on the percentage of fat (4) while the columns are the plotted values obtained for the cows grouped according to the month during which the lactation began showing the percentage of fat throughout the lactation period.

In the groups starting their lactation period during January, February, March and April, it will be seen that the percentages of fat during the summer months are considerably below the average figures for the advance of lactation. However, in September there is a rapid increase in the per cent of fat approaching very closely the average figures. During the rest of the lactation the percentages of fat exceed the average figures.

The extremely high percentages of fat during the winter months is again shown very strikingly in the groups whose lactation periods began during May, June, July and August. Starting in September and October the percentages of fat rise above the average until the following spring (April or May) when tests again tend to fall off in spite of the well known tendency for the percentage of fat to increase rapidly during the last stages of the lactation period.

In the groups beginning their lactation periods during October, November and December the seasonal variations in the per cent of fat is clearly shown. In these groups the decrease in the percentages of fat due to the season of the year occur during the

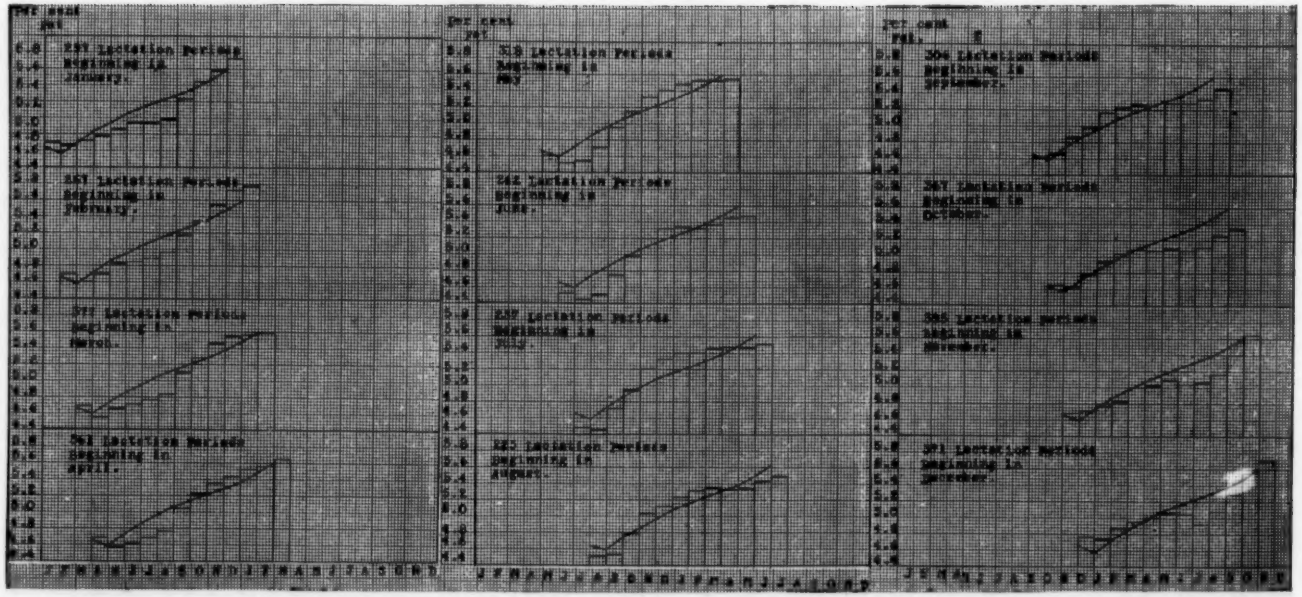

CHART I. INFLUENCE OF THE SEASON OF THE YEAR ON THE PER CENT OF FAT IN COW'S MILK. GUERNSEY

Continuous lines show influence of the stage of lactation on the percentage of fat.
Columns show seasonal variations in percentage of fat for cows grouped according to the month in which the lactation began.

latter part of the lactation period and show conclusively that the season of the year exerts a greater influence upon the percentage of fat than does the advance of lactation.

TABLE 1

Influence of the season of the year on the per cent of fat in cow's milk

Guernseys

	NUMBER OF COWS CALVING DURING											
	January 297	February 267	March 377	April 361	May 318	June 242	July 237	August 225	September 306	October 367	November 305	December 371
January	4.70											
February	4.64	4.70										
March	4.72	4.60	4.67									
April	4.79	4.70	4.55	4.65								
May	4.85	4.81	4.64	4.57	4.60							
June	4.92	4.82	4.72	4.61	4.55	4.54						
July	4.92	4.88	4.76	4.68	4.57	4.45	4.48					
August	4.98	4.97	4.83	4.77	4.65	4.51	4.43	4.49				
September	5.23	5.18	5.10	5.04	4.95	4.76	4.73	4.53	4.62			
October	5.45	5.37	5.28	5.22	5.16	4.99	4.95	4.79	4.65	4.66		
November	5.58	5.56	5.45	5.37	5.34	5.20	5.20	5.03	4.83	4.64	4.68	
December	5.72	5.73	5.54	5.43	5.41	5.32	5.32	5.14	4.99	4.79	4.70	4.78
January		5.78	5.58	5.52	5.48	5.34	5.40	5.23	5.14	4.93	4.78	4.74
February			5.68	5.55	5.54	5.32	5.39	5.30	5.21	5.04	4.81	4.84
March				5.64	5.55	5.38	5.44	5.33	5.22	5.08	5.00	4.94
April					5.58	5.44	5.48	5.31	5.20	5.07	5.01	5.00
May						5.49	5.46	5.32	5.23	5.08	5.07	5.05
June							5.51	5.42	5.23	5.07	5.02	5.03
July								5.49	5.31	5.12	5.06	4.92
August									5.45	5.24	5.15	5.09
September										5.36	5.40	5.39
October											5.64	5.58
November												5.71

The height of the column above or below the continuous line is an index of the extent of the seasonal variation of the per cent of fat. For example with the group of cows starting their lactation period in March the per cent of fat in July is 0.21 lower than the average figure. The opposite effect is best shown with the group of cows starting their lactation period in July in which

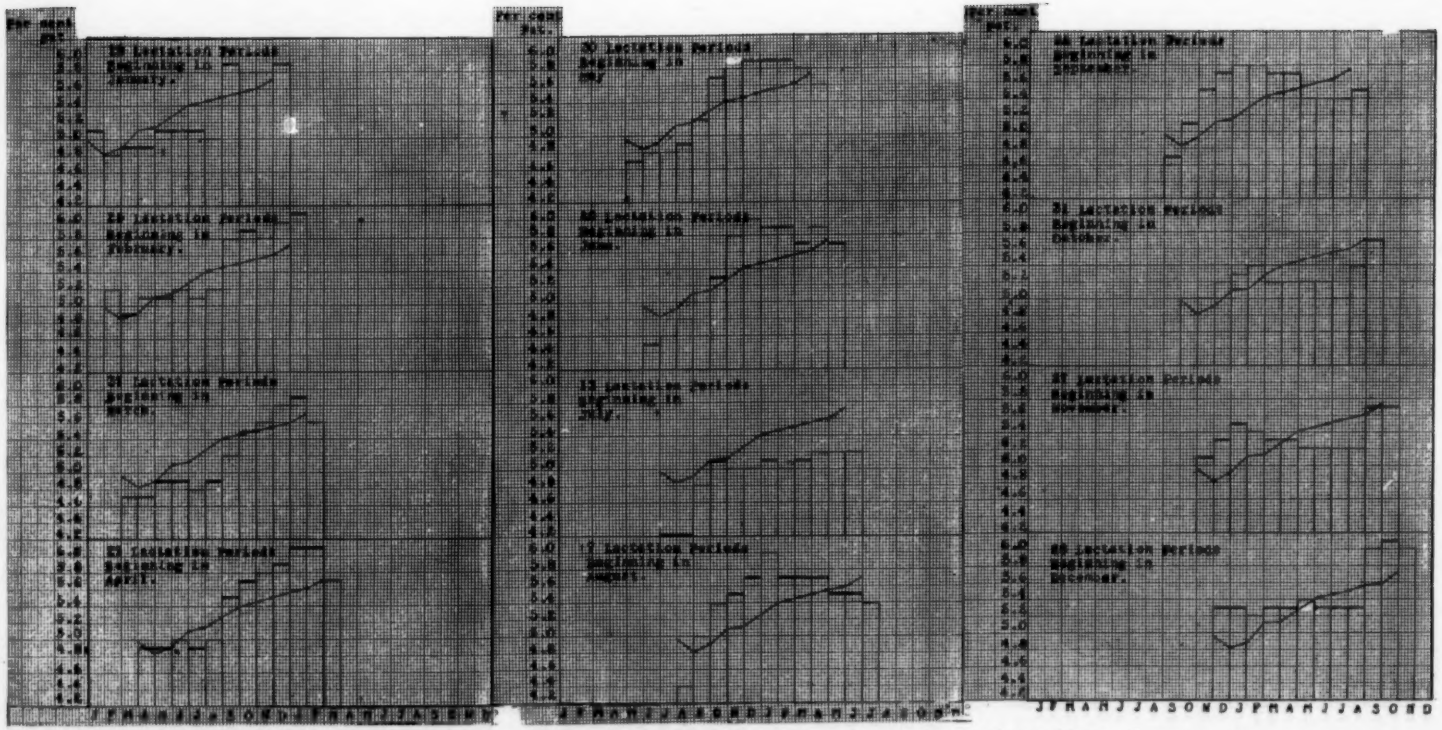

CHART II. INFLUENCE OF THE SEASON OF THE YEAR ON THE PER CENT OF FAT IN COW'S MILK. JERSEY

Continuous lines show influence of the stage of lactation on the percentage of fat.

Columns show seasonal variations in percentage of fat for cows grouped according to the month in which the lactation began.

case the per cent of fat in January is 0.34 higher than the average figures. In general it may be said that no matter when the lactation period begins, there is a tendency for the per cent of fat to rise above the average during the winter months especially

TABLE 2

Influence of the season of the year on the per cent of fat in cow's milk

Jerseys

	NUMBER OF COWS CALVING DURING											
	January 28	February 25	March 37	April 29	May 30	June 20	July 13	August 7	September 24	October 31	November 27	December 28
January	5.11											
February	4.80	5.02										
March	4.99	4.99	4.72									
April	4.99	5.15	4.71	4.83								
May	5.06	5.19	4.90	4.93	4.73							
June	5.12	5.27	4.99	5.07	4.93	4.50						
July	5.11	5.15	4.82	4.93	4.87	4.60	4.46					
August	5.03	5.20	4.96	5.14	4.97	4.80	4.46	4.43				
September	5.90	5.80	5.21	5.59	5.27	4.60	4.85	5.00	4.75			
October	5.68	5.96	5.57	5.72	5.77	5.35	5.15	5.43	5.17	5.00		
November	5.78	5.87	5.62	5.86	5.80	5.80	5.08	5.57	5.50	5.00	5.11	
December	5.88	6.07	5.82	5.97	5.93	6.00	5.08	5.71	5.75	5.26	5.30	5.32
January		6.10	5.90	6.03	5.93	5.90	5.15	6.00	5.83	5.32	5.48	5.29
February			5.61	6.10	5.90	5.90	5.08	5.71	5.88	5.39	5.41	5.21
March				5.79	5.80	5.70	5.15	5.71	5.71	5.19	5.37	5.39
April					5.60	5.90	5.23	5.71	5.61	5.22	5.37	5.36
May						5.70	5.23	5.57	5.42	5.22	5.19	5.43
June							5.31	5.57	5.46	5.22	5.22	5.36
July								5.43	5.42	5.03	5.19	5.29
August									5.50	5.39	5.15	5.32
September										5.77	5.70	6.04
October											5.67	6.11
November												6.00

December, January, and February and then to gradually decline during the spring and summer reaching the lowest point during June, July and August. These seasonal variations have been shown by Ragsdale and Brody (5) to be due to environmental temperature.

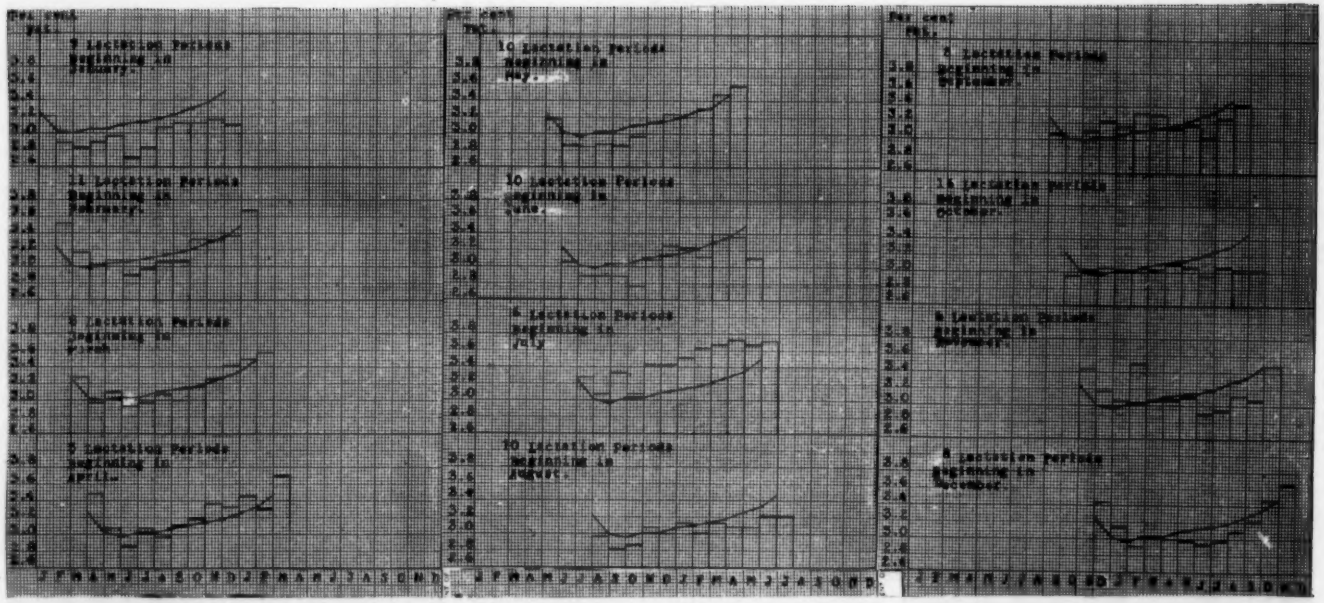

CHART III. INFLUENCE OF THE SEASON OF THE YEAR ON THE PER CENT OF FAT IN COW'S MILK. HOLSTEIN

Continuous lines show influence of the stage of lactation on the percentage of fat.
Columns show seasonal variations in percentage of fat for cows grouped according to the month in which the lactation began.

It is interesting to note the popularity of the different seasons of the year during which cows are started on official test. It is evident that both fall and spring freshening is practiced very

TABLE 3

Influence of the season of the year on the per cent of fat in cow's milk

Holsteins

	NUMBER OF COWS CALVING DURING											
	January 9	February 11	March 8	April 3	May 10	June 10	July 6	August 10	September 8	October 6	November 6	December 8
January	3.20											
February	2.87	3.53										
March	2.93	3.19	3.29									
April	2.89	3.03	2.98	3.48								
May	2.97	3.09	3.09	3.08	3.19							
June	2.71	2.90	2.93	2.86	2.87	3.05						
July	2.82	2.99	2.98	3.08	2.85	2.88	3.29					
August	3.07	3.05	3.07	2.99	2.87	2.88	3.08	2.98				
September	3.12	3.05	3.00	3.14	2.86	2.88	3.34	2.81	3.08			
October	3.12	3.34	3.18	3.20	2.97	2.76	3.08	2.87	3.01	2.95		
November	3.18	3.33	3.26	3.38	3.16	3.07	3.42	3.08	3.10	3.01	3.44	
December	3.11	3.38	3.41	3.35	3.23	3.25	3.41	3.00	3.22	3.06	3.18	3.41
January		3.67	3.49	3.44	3.20	3.23	3.50	3.14	3.17	3.03	3.07	2.16
February			3.58	3.27	3.31	3.12	3.62	3.01	3.32	3.00	3.55	2.94
March				3.71	3.47	3.40	3.66	3.15	3.25	3.05	3.04	3.03
April					3.59	3.37	3.73	3.07	3.14	3.11	3.05	3.01
May						3.11	3.66	3.07	3.19	3.06	3.10	2.99
June							3.71	3.23	3.03	2.96	2.89	2.88
July								3.22	3.25	3.06	2.91	2.98
August									3.42	3.03	3.11	3.10
September										3.04	3.09	3.21
October											3.48	3.48
November												3.67

extensively. As would be expected June, July and August appear to be the most unpopular months for cows to freshen.

From a study of the data presented it would appear that the best results would be obtained with cows calving during September or October. With cows calving during these months somewhat higher tests would be obtained during the first eight or

nine months of the lactation period when the cows are producing a maximum amount of milk.

The data for the Jersey breed is presented in table 2 and chart 2. Due to the fact that fewer records were available, as compared to the Guernsey breed, the plotted curves are not as smooth and some irregularities appear. Still the trend is the same. The groups of cows whose lactations begin in January, February and March show the decline in the percentage of fat during the summer months as compared to the average percentages of fat also the increase in the percentage of fat beginning in September and continuing during the winter months is apparent.

The decline in the percentage of fat during the following summer months is clearly shown with those groups whose lactation periods begin in September, October and November in spite of the advance of lactation.

The data for the Holstein-Friesian breed is presented in table 3 and chart 3. Since in most of the groups the number of cows was limited the seasonal variations of the percentages of fat is not as clearly shown. In the larger groups, however, the same trend is apparent as in the other breeds.

SUMMARY

Data was presented showing seasonal variations of the percentage of fat in cow's milk derived from a study of 3763 Guernsey, 299 Jersey, and 95 Holstein-Friesian yearly records.

The percentage of fat in milk when plotted follows a general curve, being lowest during the summer months, then gradually rising reaching a peak during the winter months and then again declining during the spring and summer.

When the different seasons of the year are accompanied by varying temperatures, such as ordinarily prevail throughout the greater portion of the United States, the influence upon the per cent of fat in cow's milk is greater than that of the advance of lactation.

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A METHOD FOR THE QUANTITATIVE DETERMINATION OF GELATIN IN ICE CREAMS[1]

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Since the present tentative method[2] of the Association of Official Agricultural Chemists for the detection of gelatin in milk states that a precipitate will be obtained in the presence of any considerable amount of gelatin, while smaller amounts will be indicated by a cloudiness, the implication is evident that an indication of the amount of gelatin present can be gained by observing the appearance of the precipitate.

To find out to what extent the method could be made quantitative, the turbidity produced by the addition of picric acid as directed was compared with that formed when a standard gelatin solution was treated in exactly the same way. To get a turbidity that could be well observed in the nephelometer, it was found necessary that the filtrate, after the removal of the proteins with acid mercuric nitrate, should contain from 0.025 to 0.005 per cent of gelatin. The filtrate prepared according to the tentative method was diluted until an equal volume of saturated picric acid solution gave a turbidity approximating that produced by treating similarly a 0.01 per cent solution of gelatin which had been previously tested and found to be of the highest quality. This standard gelatin solution should contain acid mercuric nitrate in as nearly as possible the same amount as the unknown solution. The results shown in table 1 were obtained by this procedure on 11 samples of ice cream mix prepared in the laboratory and containing known amounts of gelatin.

The filtrates obtained after the removal of the milk proteins with acid mercuric nitrate were practically clear when the

[1] Published by permission of the Department of Agriculture.

[2] Official and Tentative Methods of Analysis, page 229, Method No. 19.

samples contained small amounts of gelatin and were decidedly cloudy when 1 per cent or more of gelatin was present. The percentages of gelatin in the experimental ice cream (table 1) were low when 0.40 to 0.50 per cent of gelatin was present in the original sample, but were high when the samples originally contained larger amounts. A good approximation of the amount present, however, can be made by this procedure which has the advantage of being short.

TABLE 1

Estimation of gelatin in experimental ice cream by use of nephelometer

SAMPLE NUMBER	COMPOSITION OF SAMPLE			GELATIN FOUND
	Fat	Sugar	Gelatin	
	per cent	*per cent*	*per cent*	*per cent*
1	17.0	11	0.40	0.30
2	14.0	11	0.40	0.30
3	9.0	11	0.40	0.37
4	14.0	15	0.47	0.31
5	8.5	14	0.50	0.36
6	9.5		0.50	0.35
7	14.0	15	0.75	0.85
8	13.0	15	0.99	1.33
9	14.0	10	1.01	1.27
10	12.0	15	1.27	1.63
11	13.0	10	1.31	1.61

A more accurate determination of gelatin in ice cream can be made by precipitating the casein with acetic acid, throwing out the gelatin with alcohol, and dissolving the gelatin in hot water. The gelatin recovered in this way is practically free from milk protein and gives a clear solution that can be read in the polariscope for rotation and mutarotation as determined by C. R. Smith.[3,4] The detailed procedure is as follows:

PROCEDURE A

Weigh 450 grams of ice cream into a 500-cc. flask, warm to 35°, and dilute to the mark. Transfer to a larger flask and add

[3] Jour. Amer. Chem. Soc., xli (1919), 135.
[4] Jour. Ind. Eng. Chem., xii (1920), 878.

10 per cent acetic acid until the casein is coagulated, observing the volume of acetic acid added. Calculate the volume of the fat and casein (volume of fat = weight of fat in sample $\times$ 1.075; volume of casein = weight of casein in sample $\times$ 0.73, and determine the volume of serum by subtracting this from 500 plus the number of cc. of acetic acid added. If the curd does not settle readily, shake with a small amount of carbon tetrachloride, centrifuge, and decant the supernatant serum. Measure 100 cc. of the serum into a 400-cc. lipped beaker and while still warm add slowly, with stirring, 200 cc. of 95 per cent alcohol. Cool in ice water and let stand until the precipitate settles, leaving the supernatant liquid clear. At this point the precipitate may be allowed to settle over night in an ice box at as near zero Centigrade as possible. Filter on a large hardened paper in a Buchner funnel and wash with cold alcohol, two volumes of 95 per cent alcohol and one of water. Drain the precipitate, remove to a small beaker, and rub up with 50 cc. of water. Allow to soak at room temperature until the gelatin swells, then heat to about 90°C. in a hot water bath, filter, and wash with hot water. Make up to 100 cc. at 35° and polarize. Cool a portion of the solution rapidly to 15°, pour into a cold polariscope tube, let stand at 15° for twenty-four hours, and polarize again. The reading at 15°, divided by the reading at 35°, will give an indication of the jelly strength of the gelatin.[4] Calculate the percentage of gelatin in the sample from the reading at 35° from the following formula:

$$\text{Percentage of gelatin} = \frac{R \times 0.346 \times V \times 100}{W \times 2 \times \text{specific rotation}}$$

When W = weight of sample; V = volume of acetic acid serum; and R = Polariscope reading in Ventzke degrees in 2 decimeter tube.

The specific rotation of high-grade gelatin is given by Smith[3] as 141, when calculated to a moisture- and ash-free basis. Determine nitrogen in an aliquot of the gelatin solution and calulate the percentage of gelatin in the ice cream as follows:

$$\text{Percentage of gelatin} = \frac{N \times V \times 5.55 \times 100}{a \times W}$$

When W = weight of sample; V = volume of acetic acid serum; a = volume of aliquot of gelatin solution; and N = weight of nitrogen in aliquot of gelatin solution.

PROCEDURE B

When it is inconvenient to dilute the sample of ice cream to a definite volume and make a correction for the fat and casein, the following alternative procedure is proposed: Determine the reducing sugars in the ice cream according to the A. O. A. C. method for lactose in sweetened condensed milk.[5] Weigh 450 grams of the ice cream in a beaker, warm to 35°C., and add 10 per cent acetic acid until the casein is precipitated. Mix thoroughly and filter or separate the serum by centrifuging. Neutralize a 5-cc. aliquot of the serum, dilute to 50 cc., determine the reducing sugars, and calculate the total volume of the serum by dividing the weight of reducing sugars in the 450-gram sample by the weight of reducing sugars in 1 cc. of the serum. To 100 cc. of the serum add 200 cc. of alcohol and continue as outlined under procedure A.

To show that comparable results for the volume of serum were obtainable by both procedures three samples of ice cream were treated according to procedure A. Reducing sugars were determined on the ice cream and on the serum prepared under procedure A, and the volume of serum was calculated by subtracting from the total volume the volume of the fat and casein and also by dividing the weight of reducing sugars in the entire sample by the reducing sugars in 1 cc. of the serum. These results are given in table 2. The difference found in each case was 2 cc., 1.8 cc., and 1.6 cc., which is sufficiently close for practical purposes of this work.

In order to show that gelatin could be quantitatively recovered by the procedure outlined, 21 samples of ice cream mix were prepared in the laboratory and analyzed. Varying percentages of fat, milk solids not fat, sugar, and gelatin were added as shown in

[5] Official and Tentative Methods of Analysis of Association of Official Agricultural Chemists, p. 231, method no. 36.

table 4. The polariscopic observations on the samples of gelatin used in these experiments are recorded in table 3. These findings

TABLE 2

Difference between methods of calculating volume of serum

		1	2	3
Composition of ice cream mix	Fat, *per cent*	40.3	14.1	13.1
	Milk solids not fat, *per cent*		6.0	6.8
	Sugar, *per cent*	14.0	14.0	14.0
	Gelatin, *per cent*	1.00	1.00	1.00
Lactose in sample, *grams*		2.17	3.81	3.53
Lactose in 1 cc. of serum, *grams*		0.0362	0.0446	0.0409
Calculated volume of solution (procedure B)		59.9	85.4	86.3
Volume of solution by correcting for volume precipitate (procedure A)		57.9	83.6	84.7
Difference		2.0	1.8	1.6

TABLE 3

Data on samples of gelatin added to ice cream

SAMPLE NUMBER	NITROGEN	GELATIN	VENTZKE READING		READING AT 15° / READING AT 35°	SPECIFIC ROTATION AT 35°
			35°	15°		
	per cent	*grams per 100 cc.*				
A	15.7	0.50	3.6	7.2	2.00	124.6
		0.75	5.4	11.4	2.11	124.5
		0.75	5.5	11.2	2.04	126.9
		1.00	7.1	14.54	2.05	122.8
B	14.9	0.20	1.4	2.5	1.79	121.1
		0.40	2.9	5.5	1.90	125.4
		0.50	3.52	6.8	1.93	121.8
		0.60	4.08	8.3	2.03	117.6
		0.75	5.15	10.3	2.00	118.8
		0.75	5.07	10.36	2.04	116.9
		1.00	6.80	13.75	2.02	117.6
C	15.7	0.75	5.3	8.1	1.53	122.3
		1.00	6.95	11.23	1.62	120.2
D	15.7	0.50	3.45	5.10	1.48	119.4

indicate that samples A and B of gelatin are high grade in jelly strength, while sample C is poorer and sample D is the poorest,

TABLE 4

Analyses of experimental ice cream

SAMPLE NUMBER	GELATIN NUMBER	COMPOSITION OF ICE CREAM MIX				WEIGHT OF SAMPLES	VOLUME OF ACETIC ACID SERUM	VENTZKE READING*			NITROGEN IN GELATIN RECOVERED	GELATIN FOUND BY		
		Fat	Milk solids not fat	Sugar	Gelatin			35°	15°	Reading at 15° / Reading at 35°		Ventzke at 35°	Nitrogen determination	Specific rotation = 122°
		per cent	*per cent*	*per cent*	*per cent*	*grams*					*grams*	*per cent*	*per cent*	*per cent*
1†	A	3.2	7.6	11.0	0.50	300	290.6	3.70	6.70	1.81	0.0772	0.50	0.48	0.51
								3.73	6.85	1.84	0.0772	0.50	0.48	0.51
						300	299.6	3.75	6.82	1.82	0.0716	0.52	0.46	0.53
								3.50	6.50	1.86	0.0716	0.49	0.46	0.50
2	A	3.1	8.1	11.0	0.50	270	291.2	3.35			0.0760	0.50	0.52	0.51
								3.30	7.05	2.14	0.0800	0.49	0.54	0.50
3	A	3.2	7.6	11.0	0.75	300	291.0	5.20	10.40	2.00	0.1195	0.70	0.74	0.72
								4.10	7.90	1.93	0.0913	0.73	0.74	0.74
							291.0	5.05	9.40	1.86	0.1150	0.70	0.73	0.70
								5.15	9.40	1.82	0.1150	0.72	0.73	0.71
4	A	3.2	7.6	11.0	0.75	300	300.0	5.30	11.00	2.08	0.1195	0.74	0.76	0.75
								5.25	11.00	2.09	0.1180	0.73	0.75	0.74
5	A	8.3		14.25	0.75	450	477.4	4.95	10.00	2.02	0.1130	0.73	0.76	0.74
								5.05	10.30	2.04	0.1125	0.74	0.76	0.76
6	A	9.1	8.1	14.0	0.75	450	472.2	5.10	10.20	2.00	0.1135	0.74	0.76	0.76
								5.00	10.10	2.02	0.1165	0.73	0.78	0.74

7‡	A	9.0	7.5	14.0	0.75	270	282.8	5.20	10.50	2.02	0.1165	0.76	0.78	0.77
								5.30	10.80	2.04	0.1195	0.77	0.80	0.79
8†	B	3.1	7.5	11.0	0.20	300	290.9	1.40	2.50	1.79	0.0295	0.19	0.19	0.19
								1.30	2.50	1.92	0.0308	0.18	0.20	0.18
9†	B	3.1	7.3	11.0	0.40	300	291.2	2.85	5.60	1.97	0.0590	0.38	0.38	0.39
								2.75	5.30	1.93	0.0590	0.37	0.38	0.38
10	B	3.3	6.9	11.0	0.50	500	500.0	5.60	11.80	2.11	0.1400	0.40	0.47	0.40
								5.60	11.90	2.13	0.1400	0.40	0.47	0.40
11	B	3.5	7.5	11.0	0.60	300	288.6	4.40	8.80	2.00	0.0913	0.61	0.59	0.60
12	B	3.1	7.6	11.0	0.75	300	300.0	4.85	9.50	1.96	0.1080	0.72	0.72	0.69
								4.85	9.30	1.92		0.72		0.69
13	B	14.6	11.90	11.25	0.75	450	440.7	5.20	10.50	2.02	0.1214	0.74	0.80	0.72
								5.20	10.30	1.98	0.1228	0.74	0.81	0.72
14	B	12.2	9.40	11.0	1.00	100	94.5	2.80	5.50	1.96	0.0618	0.95	0.98	0.94
15	B	3.3	8.80	11.0	1.00	300	300.0	6.37	12.90	2.02	0.1400	0.90	0.98	0.90
								6.40	13.00	2.03	0.1445	0.91	0.97	0.91

* All readings are negative, the sign being purposely omitted.
† The alcohol precipitate was dissolved in water and again precipitated before making up to volume.
‡ Contained approximately 0.25 per cent Indian gum.

TABLE 4—*Continued*

SAMPLE NUMBER	GELATIN NUMBER	COMPOSITION OF ICE CREAM MIX				WEIGHT OF SAMPLES	VOLUME OF ACETIC ACID SERUM	VENTZKE READING*			NITROGEN IN GELATIN RECOVERED	GELATIN FOUND BY		
		Fat	Milk solids not fat	Sugar	Gelatin			35°	15°	Reading at 15° / Reading at 35°		Ventzke at 35°	Nitrogen determination	Specific rotation = 122°
		per cent	*per cent*	*per cent*	*per cent*	*grams*					*grams*	*per cent*	*per cent*	*per cent*
16	C	7.4	6.70	14.80	0.75	453	481.3	4.50	7.50	1.67	0.0985	0.68	0.67	0.68
								4.60	7.60	1.65	0.1025	0.69	0.70	0.69
								4.50	7.50	1.67	0.1055	0.68	0.72	0.68
17	C	7.9	7.40	11.0	1.00	450	478.8	6.30	10.10	1.60	0.1390	0.96	0.94	0.95
								6.25	10.60	1.70	0.1420	0.96	0.96	0.94
18†	D	8.8	7.70	15.0	0.50	450	474.5	2.40	3.50	1.46	0.0575	0.36	0.39	0.36
								2.40	3.60	1.50	0.0535	0.36	0.36	0.36
								2.20	3.50	1.59	0.0550	0.33	0.37	0.33
19	D	8.0	8.10	15.0	0.50	450	477.7	2.65	4.00	1.51	0.0660	0.40	0.45	0.40
								2.55	3.95	1.55	0.0630	0.38	0.43	0.38
								2.80	4.10	1.46	0.0660	0.42	0.45	0.42
20	D	8.0	8.10	15.0	0.50	450	479.7	2.40	3.50	1.46	0.0534	0.37	0.36	0.36
								2.60	3.95	1.52	0.0618	0.40	0.42	0.39
21	D	3.0	8.10	15.0	0.50	270	301.5	2.50	3.80	1.52	0.0575	0.41	0.41	0.40
								2.40	3.65	1.52	0.0575	0.39	0.41	0.38

* All readings are negative, the sign being purposely omitted.

† The alcohol precipitate was dissolved in water and again precipitated before making up to volume.

as was in fact the case. In each of the experiments reported in table 4 the volume of solution was determined by making up to a definite volume and correcting for the volume of fat and casein. The results were calculated from the Ventzke reading at 35°, using as a factor the values for each gelatin given in table 3 and again taking the average specific rotation as 122° and from the percentage of nitrogen in each sample of gelatin. The specific rotation 122° is merely the average experimental findings on samples employed and is lower than the specific rotation 141° given by Smith[6] because of the presence of moisture and ash. In other words, using the specific rotation 122° in the calculation in place of 141° gives results for gelatin on the air-dried sample. The greatest difference between the result obtained by using the observed rotation of each gelatin and the corresponding result using 122° as the average specific rotation was 0.03 per cent. In each case the percentage of gelatin calculated from the nitrogen determination was very close to that obtained from the specific rotation. Table 4 also shows the ratio of the Ventzke reading 15° to that at 35° on the gelatin recovered from the ice cream. Since these values agree with the results given in table 3, it is evident that by this method the percentage of gelatin in ice cream can be determined and its jelly strength also measured.

In all these experiments, with the exception of no. 7, gelatin was the only thickening agent used. In experiment 7 some gum appeared to be mechanically removed with the casein. The percentage of gelatin found was very slightly higher than the amount added, showing that the quantity of gum present in this experiment had little effect upon the estimation of gelatin. The effect of gum and other ice cream thickeners upon the determination of gelatin by this method remains for future investigation. Since some gums are laevo-rotatory and some dextro-rotatory and may be precipitated with alcohol, however, their absence[7] should be assured before interpreting the Ventzke reading obtained by the foregoing procedure as being due entirely to gelatin. To show that the reducing sugars have been completely

[6] Loc. cit.

[7] U. S. Dept. Agr., Bu. Chem. Bull. 116, p. 24.

removed by washing the gelatin precipitate with the dilute alcohol, their absence can be proved by testing the final gelatin solution with Fehling's solution. The presence of gelatin should be qualitatively determined by the picric acid test before making a quantitative determination by the polariscopic method.

SUMMARY

By diluting the filtrate, after removing the casein and fat with acid mercuric nitrate, according to the A. O. A. C. method for the detection of gelatin in milk, and comparing the turbidity produced by picric acid with a standard, the amount of gelatin in ice cream can be approximated.

More accurate results are obtained by precipitating the casein and fat with acetic acid, separating the gelatin with alcohol, and redissolving it in hot water. In this way the percentage of gelatin can be calculated from the nitrogen content and from the specific rotation of gelatin at 35°. The mutarotation can also be measured to show the jelly strength of the gelatin added to the ice cream. Results are shown on 21 samples of experimental ice cream mix, containing from 0.2 to 1 per cent of gelatin, using gelatins of good, medium, and poor quality.

A METABOLISM CRATE FOR CALVES AND OTHER SMALL RUMINANTS

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The metabolism crate described here is for use with calves and other small ruminants. It is felt that it will be satisfactory with sheep and goats as well as with calves as the only alterations necessary will be the grating on which the animal stands. It is also of low construction cost and easily portable. It is convenient to use in digestion and balance trials where the gaseous metabolism has not to be taken into consideration.

It consists of lower and upper parts as shown in figure 1 and there are two wheels on each side so that the upper half may be run off or on the lower section. The lower section has corner posts 4 inches square and these can be mounted on small wheels to render the moving of the crate easy.

This lower section is braced as shown in figure 1 and there is a board along each side so that it is easy to examine the animals. The collecting funnel, for the urine, of galvanized iron or zinc is attached to the lower part and the details are shown in figure 2. Running around the top of the lower section there is a 5-inch plank 1 inch thick and the funnel is so constructed that it fits into this and then runs back 4 inches on the top of the board. On top of this is a 5-inch plank, 1 inch thick which fits with the outer edge of the 6-inch plank. This 5-inch board is covered with galvanized iron on top and on the inner edge and is soldered around at this lower edge. On top of this are two runners 1 inch square to act as guards for the wheels attached to the upper section. One is set square with the outer edge of the platform and the other about $1\frac{1}{2}$ inches further in to allow room for the wheels which should be about $1\frac{1}{4}$ inches wide. The wheels are 3 inches in diameter and set in the lower part of the upper section and this gives plenty of room for them to work.

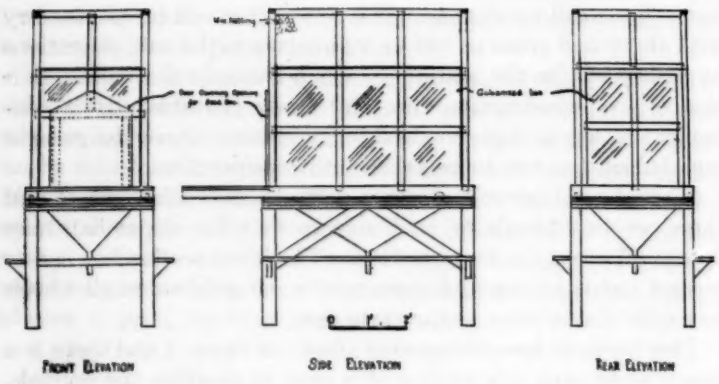
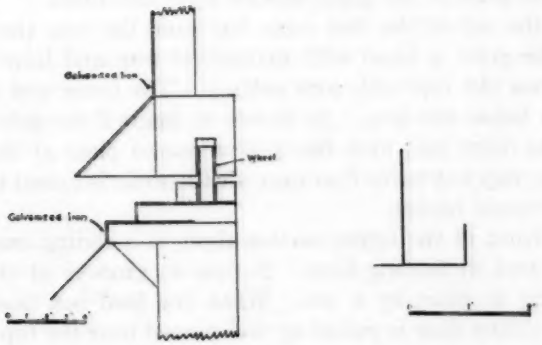


FIGURE 1



CROSS SECTION DETAIL FEED BOX CROSS SECTION

FIGURE 2

The base of the upper section, figure 2, consists of material 4 inches square and nailed to that on the inside is a quarter-sawed section of the same material. In front the basal material from the sides is carried from 2 to 3 feet forward of the crate proper, the top inch of this is taken out and platform of 1 inch material made on which to place the feed box and other material. The uprights, crossbars and top of the upper section are made of material 1 inch square as shown in figure 1. In the rear is inserted a door which reaches up to the level of the top of the second support and in its structure is the same as the other portions of the sides of the upper section to be described.

Up to the top of the first cross bar from the top, the upper part of the crate is lined with galvanized iron and from there, up and over the top with wire netting. The lower end of the netting is below the iron. As shown in figure 2 the galvanized iron comes down just over the quarter-sawed piece at the base and in this way any urine that may hit the walls is forced to drop into the central funnel.

In the front of the upper section there is a sliding iron door which is used at feeding time. It runs in grooves at the side and is kept in place by a pin. When the feed box has to be used this sliding door is pulled up and pinned near the top of the crate.

The feeding box is shown in figure 2 and is used only for a short feeding period. It is pushed in and kept in place by a pin in each side of the rear bottom portion.

The other equipment consists of a heavy galvanized iron grating which rests on the upper galvanized ledge of the lower section as shown in figure 2. The mesh, strength and size of wire, and dimensions to be used in this will be determined by the animals used. This bears the weight of the animal and the feces and urine passed through on to a screen of gauze resting on the ledge below. Here the feces are retained and the urine passes through the funnel to the collecting receptacle below. Two pins on each side prevent the upper section from moving on the track except as desired.

Additional numbers of the lower section should be provided. In fact it is better to have two lower sections for each upper. Then when the daily changing of the experimental animals occurs they can be cleaned off and moved on to the new bottoms. The feces, urine and wash water from the bottoms that have been in use are collected and all parts of the lower section thoroughly cleaned and dried. Descriptions for the use of crates of similar types are available so the details regarding the complete collection of the excreta are not required.

BACTERIA COUNTS OF MILK POWDER AS OBTAINED BY THE MICROSCOPIC METHOD[1]

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Since recent advances of the powdered milk industry have stimulated the interest of various professional groups to a degree not heretofore attained, it is not unnatural that some attention should be given to the bacterial content of this product, not only from the standpoint of numbers of living organisms present, but also from the standpoint of numbers originally contained in the milk from which it was manufactured. In fact, it has already been suggested (1) that bacterial limits should be set for liquid milk which is to be converted into powder. Irrespective of whether or not control measures are adopted which will specifically designate bacterial limits for liquid milk dried, it will be of interest to know just in how far our present methods of enumerating bacteria are of value for indicating the bacterial content of the liquid material when those methods are applied to the desiccated product. Obviously, the plate method cannot be used for this purpose, therefore the microscopic method as now used, or some modification of it, is the only alternative method which need be considered. The advantages and limitations of this method for the examination of liquid milk have been set forth in numerous publications from the New York Agricultural Experiment Station (Geneva) and need not be enumerated herein.

The adaptability of the microscopic method for the purpose under consideration,—namely, for determining the bacterial content of liquid milk used for drying by the examination of the desiccated product after reconstituting with water,—must be judged in the light of certain limitations in the method itself,

[1] Read before the Society of American Bacteriologists, Philadelphia, December 28, 1921.

and with regard to certain conditions attending the preparation of the product. Notwithstanding the statements occasionally made to the contrary, the microscopic method as now used is not accepted as being reliable for pasteurized milk. Hastings and Davenport (2) have shown that in some instances only 3 per cent of the bacteria found in raw milk can be detected by this method after pasteurization; whereas, in other samples as high as 87 per cent of those originally counted in the raw milk may be found in the pasteurized milk. These wide discrepancies between the countable bacteria before and after pasteurization seem to be due, to some extent, to the species present. From the fact that the microscopic method gives irregular results with heated milk it would seem that any attempt to apply it to reconstituted milk powder would likewise give results of doubtful value for indicating the bacterial content of the liquid milk prior to drying.

Furthermore, it is entirely possible that the degree of heat and the length of time for which the milk is exposed to high temperatures may determine to some extent the relationship between the counts obtained from the raw and heated milk. If such were found to be the case the value of the microscopic method for desiccated milks as a whole would be subject to even further limitations than those imposed by pasteurization, due to the fact that in each of the methods now commonly employed for drying milk, wide variations in temperature and period of exposure are used. Therefore, even if it were possible to state with accuracy that the microscopic method gave a true picture of the bacterial quality of the liquid milk prior to drying by a certain process, or even if it gave constant results as proved by careful comparisons showing a uniform reduction in numbers, it is doubtful whether such conclusions would be valid when the method was applied to samples dried by a different process.

Aside from these probable limitations, there still remains the further handicap of varying solubility of different samples as determined by method of manufacture and by subsequent absorption of moisture from the atmosphere during storage. This factor of insolubility tends to invalidate results by preventing proper performance of the technique rather than by directly affecting the visibility of the organisms present.

EXPERIMENTAL WORK

Even though the microscopic method may appear to have but little value for determining the bacterial content of the original liquid milk when applied to the desiccated product, certain comparisons have been made at this Laboratory for the purpose of ascertaining the actual relationship between the counts obtained from liquid milk and the counts obtained from the same milk after drying by the Just hot-roller process. The particular object of these comparisons was to determine whether this method could be relied upon to indicate gross differences in bacterial content of milk dried each day at different factories, thereby furnishing a basis upon which a control system might be established which would be at least applicable to milk powder made by the Just process.

While there is some doubt as to the inherent value of statements which designate a definite number of bacteria per cubic centimeter, there are certain instances which demand that such expressions be used, and that the numbers recorded be determined as accurately as the particular circumstances may dictate. In making comparisons of bacterial counts from milk by the microscopic method before and after drying, it has been necessary to obtain figures designed to show the actual difference in the number of bacteria found in the respective samples. These comparative counts were obtained in the following manner:

Convenient amounts of the experimental liquid milk (400 to 500 pounds) were placed in an especially devised tank which was attached directly to the feed pipe of a Just process drying machine. After sampling, the milk was turned on and when it had reached the normal level in the trough formed by the revolving cylinders and drying conditions had become normal in other respects, samples of the film of dry milk solids were taken immediately after being cut from the cylinders, this film being subsequently reduced to a fine powder. Soon after the samples were taken duplicate smears from both liquid and dried products were made for microscopic examination. The technique regularly employed for liquid milk was used, the samples of powder having first been diluted with water to give the same concentration of milk solids

as was found in the natural milk. One hundred microscopic fields were examined on each of the duplicate smears by two different analysts. Groups of bacteria consisting of one or more organisms as well as total number of organisms were counted. The results obtained from these examinations are shown in table 1.

From the results given in table 1 it is to be noted that there is a lower number of bacteria in all samples of reconstituted milk than was found in the original liquid milk in all cases except one. Excluding this single sample, the number which could be found in the desiccated milk varied from 30 per cent to 78 per cent of the total found in the original liquid milk; the average from all samples being 45.3 per cent. These results are in fair agreement with those found by Hastings and Davenport (2) from pasteurized milk, and are particularly significant in that they were obtained from a product dried by a process in which the milk is exposed to a temperature above the boiling point for a few seconds.

In reviewing the counts for groups of bacteria consisting of one or more organisms, a remarkably close agreement is found in four of the samples. In two of the samples the group count is higher in the reconstituted milk than in the natural liquid milk. In the remainder of the samples the group count is materially lower in the reconstituted milk. The only interpretation which can be made from these results, is that, there is a tendency for clumps of bacteria to be broken up during the drying process with the consequent result that in the desiccated product there are more organisms existing singly or in small groups of 2, 3, or 4 organisms than were present in the original milk. This difference is readily illustrated by comparing the average size of groups in the two samples. Such a comparison shows that in every sample the average size of the groups is smaller in the desiccated milk than in the natural milk. The average number of organisms per group from all samples of natural milk is 2.7, whereas the average for the desiccated samples is 1.7.

If the microscopic method were to be applied to reconstituted dry milk samples as a routine procedure for determining the general grade of milk dried, or for obtaining information sup-

TABLE 1

Microscopic counts of liquid and dry milk

SAMPLE	ANALYST	SMEAR	NATURAL LIQUID MILK (BACTERIA PER CUBIC CENTIMETER)		RECONSTITUTED DRY MILK (BACTERIA PER CUBIC CENTIMETER)	
			Total count	Group count	Total count	Group count
I	A	1	1,680,000	820,000	1,250,000	500,000
		2	2,750,000	980,000	1,060,000	560,000
	B	1	3,100,000	1,500,000	1,500,000	1,050,000
		2	4,700,000	1,950,000	2,450,000	1,350,000
Average			3,057,000	1,312,500	1,565,000	865,000
II	A	1	2,000,000	550,000	770,000	370,000
		2	1,900,000	450,000	1,410,000	510,000
	B	1	3,500,000	1,500,000	2,000,000	1,300,000
		2	5,000,000	1,850,000	2,300,000	2,000,000
Average			3,100,000	1,087,000	1,870,000	1,045,000
III	A	1	8,950,000	3,450,000	9,450,000	6,300,000
		2	11,200,000	5,200,000	11,250,000	6,300,000
	B	1	10,250,000	4,100,000	11,650,000	7,100,000
		2	14,900,000	4,950,000	13,500,000	7,600,000
Average			11,325,000	4,450,000	11,460,000	6,825,000
IV	A	1	3,610,000	1,400,000	1,560,000	1,130,000
		2	3,840,000	1,330,000	2,270,000	1,170,000
	B	1	4,300,000	1,450,000	2,900,000	1,150,000
		2	4,400,000	1,250,000	3,000,000	1,300,000
Average			4,037,500	1,357,000	2,432,500	1,187,500
V	A	1	36,640,000	19,560,000	9,510,000	6,630,000
		2	42,850,000	21,770,000	17,730,000	11,020,000
	B	1	35,000,000	13,800,000	9,700,000	5,400,000
		2	39,600,000	16,500,000	14,350,000	8,550,000
Average			38,522,000	17,907,000	12,822,500	7,900,000
VI	A	1	166,600,000	78,500,000	44,600,000	26,990,000
		2	147,100,000	67,600,000	46,730,000	26,820,000
	B	1	154,500,000	52,500,000	56,400,000	31,600,000
		2	160,000,000	46,700,000	52,200,000	26,800,000
Average			157,050,000	61,325,000	49,982,500	28,052,500

TABLE 1—*Continued*

SAMPLE	ANALYST	SMEAR	NATURAL LIQUID MILK (BACTERIA PER CUBIC CENTIMETER)		RECONSTITUTED DRY MILK (BACTERIA PER CUBIC CENTIMETER)	
			Total count	Group count	Total count	Group count
VII	A	1	2,930,000	1,470,000	1,600,000	770,000
		2	3,600,000	1,730,000	1,370,000	830,000
	B	1	5,650,000	2,750,000	1,100,000	550,000
		2	5,000,000	1,950,000	1,200,000	650,000
Average			4,295,000	1,975,000	1,317,500	700,000
VIII	A	1	3,130,000	1,570,000	800,000	570,000
		2	4,150,000	1,720,000	1,600,000	940,000
	B	1	3,850,000	1,750,000	1,100,000	750,000
		2	4,350,000	2,050,000	2,000,000	11,200,000
Average			3,870,000	1,772,500	1,375,000	865,000
IX	A	1	17,700,000	9,140,000	19,000,000	11,750,000
		2	17,600,000	9,590,000	9,500,000	6,360,000
	B	1	14,350,000	6,950,000	8,880,000	5,500,000
		2	15,550,000	7,150,000	13,650,000	8,400,000
Average			16,300,000	8,207,500	12,807,500	8,002,500
X	A	1	3,280,000	810,000	3,150,000	1,090,000
		2	4,760,000	1,120,000	2,150,000	670,000
	B	1	3,500,000	850,000	1,950,000	700,000
		2	7,950,000	1,200,000	2,100,000	800,000
Average			4,872,500	995,000	2,337,000	815,000
XI	A	1	6,650,000	1,910,000	3,300,000	1,150,000
		2	6,710,000	2,160,000	3,080,000	1,100,000
	B	1	8,450,000	2,000,000	3,950,000	1,650,000
		2	8,750,000	2,450,000	5,150,000	1,850,000
Average			7,640,000	2,130,000	3,870,000	1,437,000
XII	A	1	46,270,000	12,120,000	35,460,000	20,300,000
		2	42,820,000	11,120,000	27,890,000	16,500,000
	B	1	75,700,000	13,400,000	51,100,000	33,800,000
		2	82,000,000	15,200,000	51,100,000	29,500,000
Average			61,697,000	12,955,000	41,387,000	25,025,000
Average all samples			26,311,000	9,622,000	11,930,000	6,892,000

plementary to bacterial counts regularly determined on milk delivered by patrons, it would be impracticable to spend the time necessary to count the bacteria in 200 microscopic fields as was done with the samples recorded in table 1. In order to ascertain the reliability of counts obtained by the examination of a minimum number of fields, the twelve samples from which 100 fields had been counted on duplicate preparations were examined after an interval of six months without the analyst having knowledge of the results obtained from the detail counts. In the

TABLE 2

Comparisons of results obtained from examination of 200 microscopic fields and 10 microscopic fields

SAMPLE	NATURAL LIQUID MILK (BACTERIA PER CUBIC CENTIMETER —GROUP COUNT)		RECONSTITUTED DRY MILK (BACTERIA PER CUBIC CENTIMETER —GROUP COUNT)	
	200 fields	10 fields	200 fields	10 fields
I	1,312,000	750,000	865,000	650,000
II	1,087,000	1,025,000	1,045,000	900,000
III	4,450,000	4,275,000	6,825,000	4,975,000
IV	1,357,000	1,125,000	1,187,000	1,250,000
V	17,907,000	9,875,000	7,900,000	6,625,000
VI	61,325,000	54,500,000	28,052,000	38,625,000
VII	1,975,000	2,225,000	700,000	1,300,000
VIII	1,772,000	2,350,000	865,000	1,400,000
IX	8,207,000	8,675,000	8,002,000	9,625,000
X	995,000	700,000	815,000	750,000
XI	2,130,000	1,975,000	1,437,000	1,450,000
XII	12,955,000	6,400,000	25,025,000	36,000,000

second examination only groups of bacteria were counted in 5 fields of each of the duplicate preparations. The average results of these examinations compared with those obtained by examining 100 fields from duplicate preparations are shown in table 2. These results would seem to indicate that for the purpose of determining the relative bacterial content of either raw or desiccated milk, there is very little to be gained for control purposes by counting a large number of fields.

In order to secure further information showing the difference in bacterial count of raw liquid milk and reconstituted dry milk

as determined by the practicable method of counting groups in 5 microscopic fields, additional samples were obtained daily for a period of one month and handled in all respects as would be

TABLE 3

Comparisons of number of groups of bacteria in natural and desiccated milk as determined by practicable methods of examination

SAMPLE	NATURAL LIQUID MILK (BACTERIA PER CUBIC CENTIMETER)	RECONSTRUCTED DRY MILK (BACTERIA PER CUBIC CENTIMETER)
1	2,040,000	840,000
2	1,680,000	900,000
3	3,300,000	1,320,000
4	1,800,000	1,080,000
5	5,100,000	1,140,000
6	2,160,000	540,000
7	2,270,000	840,000
8	3,180,000	660,000
9	4,680,000	1,860,000
10	11,400,000	3,420,000
11	8,100,000	5,400,000
12	3,600,000	1,380,000
13	2,400,000	960,000
14	5,460,000	1,920,000
15	3,300,000	960,000
16	2,220,000	840,000
17	2,640,000	1,080,000
18	1,800,000	960,000
19	5,520,000	1,740,000
20	1,980,000	960,000
21	3,000,000	1,800,000
22	7,800,000	3,000,000
23	1,560,000	480,000
24	6,600,000	3,000,000
25	16,000,000	6,600,000
26	4,980,000	1,800,000
27	2,500,000	2,400,000
28	4,800,000	3,120,000
29	15,000,000	5,040,000
30	3,600,000	1,920,000
Average	4,649,333	1,932,000

done under routine control conditions. The results of these comparisons are shown in table 3 from which it will be noted that the same general conditions are found as were obtained from

the samples which have already been recorded. These samples, however, contain fewer bacteria than the others and there was an absence of large groups of organisms in the natural milk. This undoubtedly accounts for the fact that none of these samples showed an increase in the group count from the reconstituted milk. The average groups of bacteria counted in the desiccated samples amounted to 41.4 per cent of the number found in the original milk. The close relationship between this figure and that obtained from the samples in which 200 fields were counted tends to strengthen the conclusion that from milk of normal flora, on an average something less than 50 per cent of the total number of bacteria can be detected by the microscope after drying by the Just process.

Variability of microscopic counts from liquid and dried milk

It is not considered that this investigation would be complete without attempting to show the relative accuracy of the microscopic method for natural milk and for reconstituted dry milks in so far as the technique alone is concerned. Since there are certain factors which might cause variations in the counts from the same sample of reconstituted milk, which are not found in natural milk it has seemed advisable to determine the coefficient of variability from samples of liquid milk and samples of milk powder made by different processes in order that the constancy of this factor for each product may be known. This information would also be of value in interpreting the difference obtained between the counts from natural and reconstituted milk, in that, if the same coefficient of variability should be found from both products, the difference in counts such as those already shown in the foregoing tables would be directly attributable to the disintegration of certain organisms or to lack of ability to take the stain rather than to other factors such as, loss of organisms resulting from failure of the desiccated product to adhere to the slide; invisibility due to poor solubility of the powder; lack of uniform thickness of milk solids; or to other interfering factors which might result from desiccation and subsequent reconstitution.

Breed and Stocking (3) have shown that a coefficient of variability as low as 11.7 may be obtained by the microscopic method for group counts made from milk artificially inoculated in order to eliminate the presence of irregular groups containing a large number of organisms. The high degree of accuracy represented by this figure cannot, however, be accepted as applicable to samples of natural milk which may contain single organisms and irregular-sized groups in varying ratios. In order to ascertain the coefficient of variability from milk with natural groups, the group count was obtained on 9 different samples, 10 different preparations being made from each sample. The groups of

TABLE 4

Variations in group counts obtained from normal milk

SAMPLE	AVERAGE BACTERIA PER CUBIC CENTIMETER	STANDARD DEVIATION	PROBABLE ERROR	COEFFICIENT OF VARIABILITY
1	18,000	±11,400	7,200	61.2
2	78,000	±49,800	33,000	63.8
3	90,000	±45,000	30,600	52.8
4	66,000	±34,000	22,800	51.8
5	5,880,000	±3,120,000	2,100,000	53.0
6	5,220,000	±2,640,000	1,740,000	50.5
7	93,000,000	±14,880,000	10,000,000	16.0
8	49,800,000	±13,800,000	9,300,000	29.0
9	40,200,000	±16,380,000	11,040,000	40.7
Average				46.3

bacteria were counted in 100 fields on each of the ten smears from each sample. The average count for each sample, the standard deviation, probable error and coefficient of variability are shown in table 4.

Since the samples in table 4 represent wide variations in bacterial quality, it is believed that the average coefficient of variability of 46.3 represents very closely the degree of accuracy which may be expected from the microscopic count of normal samples of liquid milk.

In order that the coefficient of variability from samples of reconstituted milk might be compared with that obtained from milk with natural bacterial groups, samples of powder made by

different processes and with wide variations in bacterial content were selected and the group counts determined. The plan used for obtaining these counts follows:

Each of the samples of dry milk were divided into four or five parts and the necessary amount of water added to each portion to reconstitute to the concentration of the original liquid milk. From each of these separate samples several preparations were made. Not less than 5 microscopic fields were examined from each preparation. The average result from the 5 fields was taken

TABLE 5

Bacteria counts and coefficient of variability obtained from different samples of milk powder

SAMPLE NUMBER	PROCESS OF MANUFACTURE	AVERAGE BACTERIA PER CUBIC CENTIMETER (GROUP COUNT)	COEFFICIENT OF VARIABILITY
1	Hot roller (Just)	7,320,000	36.0
2	Hot roller (Just)	11,370,000	23.6
3	Hot roller (Just)	14,540,000	39.4
4	Hot roller (Just)	199,900,000	22.0
5	Hot roller (Just)	42,900,000	13.6
6	Hot roller (Just)	295,000	68.4
7	Hot roller (Just)	89,000	104.9
8	Hot roller (Just)	15,732,000	20.9
9	Kunick modified	88,956,000	5.9
10	Kunick modified	2,580,000	17.8
11	Kunick modified	1,836,000	21.3
12	Spray	9,780,000	15.3
13	Spray	7,656,000	28.8
14	Spray	2,764,000	40.2
Average			32.7

as the count for the sub-sample. The results from the different sub-samples were used for determining the coefficient of variability for that particular sample of powder. In table 5 is shown the average bacteria per cubic centimeter (group count) of reconstituted milk and the coefficient of variability as determined from the counts as already explained. From the results shown in this table it is to be noted that the average coefficient of variability from determinations made upon 14 different samples is 32.7. This figure indicates even a greater uniformity of results than is

to be expected from natural liquid milk providing the coefficient of varibility of 46.3 from such samples is to be accepted as a criterion of uniformity. It is significant that there is a relatively close agreement in the coefficients of varibility from each type of milk powder when the results are over a million bacteria per cubic centimenter.

CONCLUSIONS

The following conclusions may be drawn from the results reported herein:

1. There is a decrease in the number of bacteria which can be detected by the microscopic method in reconstituted milk powder dried by the Just process. The decrease in total numbers as determined by examining 100 fields from duplicate preparations is not constant; variations from 30 per cent to 78 per cent of the number originally present in the natural milk were found in the samples of reconstituted milk. The average number found from all samples of reconstituted milk was 45.3 per cent of those counted in the natural milk.

2. There is a tendency for groups of bacteria existing in natural milk to be broken up during the drying process. Group counts, therefore, may not be reduced in the same proportion as the total count. Relationship between group counts from natural and reconstituted milk powder depends upon the prevalence of large groups in the natural milk. In some instances the group count from reconstituted milk is higher than the group count from natural milk. From samples of natural milk which do not contain excessive numbers of large groups, there was a reduction in group count very similar to the reduction in total count.

3. For practical routine examination of natural or reconstituted milk for control purposes in which it is desired to grade samples into three or four classes, the average count obtained from 10 microscopic fields gives practically as satisfactory results as does the examination of 200 fields.

4. The microscopic method as applied to samples of reconstituted milk gives results which are fully as uniform as those obtained from natural milk when the adaptability of the method

for discerning visible organisms is alone concerned. This statement does not apply to old samples of powder which have become insoluble and thus prevent proper performance of the regular technique.

5. Microscopic examinations of milk powder made by the hot roller process are justified as a partial control measure for commercial laboratories in which it is desired to ascertain the relative bacterial content of the milk dried. The results however, must be interpreted with due recognition of the fact that a certain percentage have disappeared during the process; the results reported herein show that the average decrease has amounted to approximately 50 per cent.

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